Table 12b: Haematological diagnosis of the T cell subgroup.

#Pt	Sex	Age	Eth Grp	Sp.	Blast (%)	TWBC (cells/ul)	Pathological Report: Cytochemistry Tests Perox.	PAS	APhos	Diagnosis
NC				PB	0	5,000-8,000				normal control
T-1	F	2	C	BM	95	352,000	-	fine+coarse	ND	L2**
T-2*	M	34	M	PB	87	68,200	-	+	ND	ALL
T-3	M	38	M	PB	46	210,600	few	-	ND	AML
T-4	M	46	C	PB	92	65,300	-	-	polar	NHL
T-5	M	68	C	PB	88	325,600	NF	NF	NF	?
T-6	F	27	C	PB	46	9,700	-	-	polar	L1(T)
T-7	F	39	C	PB	33	87,800	-	fine gran.	ND	L2**
T-8	M	17	M	PB	54	29,300	-	coarse	ND	L1
T-9	M	33	O	PB	41	85,700	60%	granular	ND	M4(? + lymphoma)
T-10	M	72	C	PB	88	79,600	-	block	NF	L1
T-11*	M	2	C	PB	NF	NF	NF	NF	NF	T
T-12	M	23	I	PB	35	108,300	NF	NF	NF	NHL
T-13	M	24	M	BM	80	10,100	-	fine	ND	NHL
T-14	M	38	C	PB	88	13,100	few	-	+	M4
T-15	M	4	M	PB	43	14,000	-	block	ND	L1
T-16	M	14	I	BM	85	118,000	-	coarse gran. + block	ND	L2
T-17	M	19	I	BM	71	107,700	-	granular	ND	NHL**
T-18	M	13	C	PB	68	29,900	-	+	ND	NHL
T-19	M	4	M	BM	86	373,200	-	granular	+	L2(Rlps)**
T-20	M	4	M	PB	97	76,420	-	-	polar	L1
T-21	F	7	O	PB	93	325,800	-	-	polar	T-ALL
T-22	M	16	M	PB	93	198,900	-	-	ND	L2
T-23	M	15	M	PB	79	233,000	-	-	+	T-ALL**
T-24	M	14	I	PB	67	54,500	-	-	+	ALL
T-45	M	18	I	BM	90	60,600	-	-	+	L2**
T-26	M	18	M	PB	79	99,100	-	coarse gran.	ND	NHL
T-27	M	25	M	BM	>80	24,200	-	gran.+block	polar	ALL(Rlps)
T-28	M	16	C	PB	NF	84,800	NF	NF	NF	?
T-29	M	4	C	BM	85	614,000	-	-	polar	T-ALL
T-30	M	32	I	PB	17	22,400	-	-	+	ALL
T-31	M	32	C	BM	>90	22,300	-	coarse gran.	ND	NHL
T-32	M	2	M	BM	19	30,300	-	coarse gran. + block	ND	L1**
T-33	F	3	C	PB	54	29,300	NF	NF	NF	NHL
T-34	M	61	C	PB	33	47,500	— dry tap —			NHL
T-35	F	7	C	BM	40	4,900	-	-	ND	L2
T-36	M	8	M	BM	>90	294,300	-	-	ND	L1
T-37*	M	15	C	PB	NF	4,200	NF	NF	NF	T-ALL

wk - weak NC - Normal Control

Table 6: Distribution of the total number of patients admitted to UHKL between September 1988 and September 1990.

Ethnic	Sex	(1 yr.	1-11yrs.	)=12yrs.	Total	Ratio (M:F)	%
Malay	Male	2,268	1,895	5,359	9,522	1.07:1	
	Female	1,741	1,380	5,809	8,930		
	Subtotal	4,009	3,275	11,168	18,452		32.2
Chinese	Male	886	1,932	9,358	12,176	1.07:1	
	Female	756	1,240	9,421	11,417		
	Subtotal	1,642	3,172	18,779	23,593		41.1
Indian	Male	749	1,022	6,214	7,985	1.24:1	
	Female	680	663	5,095	6,438		
	Subtotal	1,429	1,685	11,309	14,423		25.1
Others	Male	37	48	361	446	1.04:1	
	Female	17	24	387	428		
	Subtotal	54	72	748	874		1.5
Total		7,134	8,204	42,004	57,342	1.11:1	

The Statistics Department reported an average (based on three years: 1988, 1989 and 1990) of 505 discharges and 58 deaths from leukaemia every year. Adults made up 238 discharges and 36 deaths, while paediatric wards discharged 267 and recorded 22 deaths.

Between September 1988 and September 1990, 433 bone marrow aspirates were performed by the Haematology Department of UHKL. These consisted of new and relapse cases of ALL, AML, CLL and patients with non-Hodgkin's lymphoma suspected of having marrow infiltration (NHL). The breakdown according to sex, age and ethnic group is shown in Table 7. A higher number of paediatrics were diagnosed with acute leukaemia (A:P ratio of 1:1.3), while the M:F ratio was 1.2:1.

ALL was the most common leukaemia admitted, forming 60.5% (262/433) of the total number. This was followed by AML (30.7%, 133/433), NHL (7.8%, 34/433) and CLL (0.9%, 4/433) (Table 7). Distribution of races showed that the majority of acute leukaemia patients were Chinese (55.2%, 237/429), followed by Malays (32.9%, 141/429), Indians (10.7%, 46/429) and Others (1.2%, 5/429) (Table 7). A comparison with the total number of patients admitted (Table 6) revealed 1.00% (237/23,593) of Chinese, 0.76% (141/18,452) of Malays, 0.32% (46/14,423) of Indians and 0.57% (5/874) of Others suffered from leukaemia.

More paediatric patients suffered from ALL, 72.5% (190/262)

Table 7: Approximate incidence of leukaemia among the ethnic groups and the adult and paediatric patients in UHKL.

Ethnic	Sex	ALL A	ALL P	NHL A	NHL P	AML A	AML P	CLL A	CLL P	Total	% Ethnic
Malay	Male	9	47	2	2	15	5	-	-	80	
	Female	6	37	1	1	10	6	-	-	61	
	Subtotal	15	84	3	3	25	11	-	-	141	32.6
Chinese	Male	21	57	11	3	23	11	2	-	128	
	Female	27	36	4	5	30	9	2	-	113	
	Subtotal	48	93	15	8	53	20	4	-	241	55.7
Indian	Male	7	9	3	1	6	2	-	-	28	
	Female	1	4	1	-	9	3	-	-	18	
	Subtotal	8	13	4	1	15	5	-	-	46	10.6
Others	Male	1	-	-	-	3	-	-	-	4	
	Female	-	-	-	-	-	1	-	-	1	
	Subtotal.	1	-	-	-	3	1	-	-	5	1.2
Total		72	190	22	12	96	37	4	-		
%		16.6	43.9	5.1	2.8	22.2	8.5	0.9	-		
Grand Total		262		34		133		4		433	
%		60.5		7.8		30.7		0.9			

A - Adult P - Paeds.

while adults formed the majority of patients in AML, (72.2%, 96/133), NHL (64.7%, 22/34) and CLL (100.0%, 4/4) cases (Table 7).

3.2 IMMUNOPHENOTYPING (IP) AND HAEMATOLOGICAL DIAGNOSIS

Bone marrow aspirations were done on the majority of patients suspected of leukaemia. The few missed were on account of patient's sudden death or refusal. Samples were considered positive if at least 10-20% blasts were stained (Foon and Todd, 1986).

Approximately, 47% (138/296) of ALL/NHL, 30% (40/133) of AML and 75% (3/4) of CLL cases sent to the Haematology Department were immunophenotyped (data not shown). These made up a total of 181 (41.8%) cases of leukaemia from UHKL, while an additional eight patients' samples were collected from other medical centres (*) (Table 8). Table 8 also shows the breakdown of leukaemia cases based on phenotypic subgroups.

3.2.1 Phenotypic Subgroups

The total white blood cell count (TWBC), type of specimen (Sp) and the percentage of blast at the time of sample collection were obtained from the Haematology Department. Control was carried out on normal blood samples collected at the Blood Bank, UHKL.

Pui et al. (1993) divided ALL patients into four age groups

Table 8: Breakdown of the total number of leukaemia cases collected for immunophenotyping based on phenotypic subgroups.

	"Null"ALL	C-ALL	B-ALL	T-cell	AML	CLL	Total
Adult							
Male	4	8+1	2	22+2	10+1	2	48+4
Female	2	15	1+1	2	18+2	1	39+3
Subtotal	6	23+1	3+1	24+2	28+3	3	87+7
Paeds							
Male	9	25	3	6+1	6	-	49+1
Female	4	28	3	4	6	-	45
Subtotal	13	53	6	10+1	12	-	94+1
Total	19	76+1	9+1	34+3	40+3	3	181+8
%	10.5	45.0	5.0	18.8	22.1	1.6	

+ cases not admitted in UHKL.

(Table 4). Leukaemia cases here were also grouped similarly, with the exception of the pre-B subgroup. Cells of the pre-B subgroup mature from early pre-B, CD10+ cells and are still CD10 positive, thus they were grouped as one. Values were shown in Table 9. M:F ratios of AML cases were added for comparison.

Table 9: Percent distribution of ALLs phenotyped in UHKL

Phenotype	Infants (<1.5yr n=6) (M : F)	Children (1.5-10yr n=72) (M : F)	Adolescents (>10yr n=14) (M : F)	Adults (>15yr n=46) (M : F)
Early pre-B				
CD10+	33.3 (2:0)	69.4 (24:26)	57.1 (4:4)	34.8 (4:12)
CD10-	33.3 (2:0)	12.5 (7:2)	21.4 (1:2)	10.9 (3:2)
T	0.0 (0:0)	12.5 (5:4)	21.4 (3:0)	47.8 (20:2)
B	33.3 (0:2)	5.6 (2:2)	0.0 (0:0)	6.5 (2:1)
AML	(1:1)	(3:4)	(2:4)	(9:14)

M:F - male:female

3.2.1.1 Acute Lymphoblastic Leukaemia

CD19+, CD20+ or CD19+ and CD20+ cases were classified as B lineage cells. A total of 104 cases of ALL were B cells, forming 75.4% (104/138) of samples collected from UHKL (Table 8). They were categorized as null ALL (or early pre-

B, Calla-), C-ALL (early pre-B, Calla+ or pre-B cells) and B-ALL (Table 8).

Null-ALL

A total of 19 patients presented with the null-ALL subtype (Ia+B4+). Most of the cases showed high positivity for B4 and Ia in good agreement to the percentage of blast cells in the sample. However, a lower percentage of Ia compared to that of B4 was also seen (for example N-5, 6, 12, 15 and 19). These appeared to be similar to results observed in the C-All subgroup and were not due to bad preparation of the sample (see below). Mature B cell markers, B1 and SmIg (surface membrane immunoglobulin), were consistently low as were the T cell marker (CD7) and myeloid markers, My7 and My9 (Table 10).

The Haematology Department carried out cytochemistry tests and reported on the FAB morphology of cells in the bone marrows received. Cytochemistry tests included peroxidase (Perox), periodic acid Sciff (PAS), acid phosphatase (APhos), specific esterase (SE) and non-specific esterase (NSE). Half of the null-ALL cases were positive for PAS. All except one were of granular positivity with two having an additional block positivity. Acid phosphatase was not done routinely, but one case, N-14, showed polar positivity and was diagnosed as a T-cell ALL with a L2 morphology. IP results, however, were negative for T2, T11, T4 and T8.
Of 19 patients, only 6 (31.6%) were females. Most significant

Table 10: Surface marker expression, haematological diagnosis and biological features of the Null-ALL subgroup.

							Pathological Report				Immunophenotyping								
							Cytochemistry Tests				Surface Marker Expression (%)								
#Pt	Sex	Age	Ethn	Sp.	Blast (%)	TWBC (cells/ul)	Perox.	PAS	APhos	Diagnosis	Ia	B4	Calla	B1	Smig	T2	My7	My9	Others
NC				PB	0	5,000-8,000				normal control	34	14	-	15	28	11-68	10	14	
N-1	M	1/12	M	PB	98	17,900	-	-	ND	L1	62	72	4	10	ND	5	3	4	-
N-2	M	4/12	C	BM	>90	95,100	-	granular	ND	ALL**	90	91	-	ND	10	2	3	3	-
N-3	F	2	C	BM	90	7,500	-	granular	ND	L2	84	84	-	ND	3	-	-	8	-
N-4	M	3	C	BM	80	7,200	-	granular	ND	Pre-B**	76	72	-	1	10	9	-	-	-
N-5	M	3	C	BM	70	22,000	-	granular	ND	ALL**	69	89	-	ND	18	7	ND	ND	My4-
N-6	M	3	C	BM	>80	12,800	-	-	ND	L1	27	84	-	ND	ND	ND	ND	ND	-
N-7	M	4	M	PB	58	39,900	-	-	ND	ALL**	68	73	-	ND	ND	-	5	-	
N-8	M	5	C	PB	0	6,600	ND	ND	ND	Partially Treated ALL**	43	40	3	24	3	23	8	-	-
N-9	M	8	C	PB	72	25,600	NF	NF	NF	NF	92	79	1	ND	5	4	-	-	-
N-10	F	8	C	PB	88	64,000	-	fine gran	ND	L1	77	72	3	5	ND	3	-	-	-
N-11	M	9	M	BM	>90	7,000	-	fine gran + block	ND	L1	78	83	-	-	3	-	-	-	-
N-12	F	11	C	BM	>90	1,400	-	-	ND	L1,10%L2	-	39	-	ND	3	-	-	-	-
N-13	F	11	C	PB	53	5,900	+	block gran	ND	ALL	62	64	5	ND	11	21	-	-	-
N-14	M	14	C	PB	80	110,600	-	fine gran + block	polar	L2(T)	76	95	-	ND	5	-	-	-	T11-T4-T8-
N-15	F	18	C	BM	^13	15,800	NF	NF	NF	NF	54	84	-	ND	15	15	12	ND	-
N-16	F	19	M	BM	70	1,000	-	-	-	NHL	ND	68	-	5	5	ND	ND	ND	-
N-17	M	23	C	PB	58	4,900	-	-	-	L1	ND	70	3	ND	-	15	-	-	-
N-18	M	38	I	BM	90	15,000	-	-	ND	L2	66	72	-	ND	ND	-	7	7	-
N-19	M	48	C	PB	>40	86,000	-	-	ND	L1	25	88	-	6	10	-	-	-	B2-

** Cases where immunophenotyping results were included in bone marrow report.
^ Percentage blast in PB

ND - not done
BM - bone marrow
NC - Normal Control

NF - not found
PB - peripheral blood
- - negative value

Ethn - Ethnic
M - Malay
I - Indian
C- Chinese
O - Others

TWBC - Total White Blood Cell Count

was the much higher ratio of males among the young. Both infants were males and the M:F ratio among children was 7:2. The M:F ratios among adolescent and adults were 1:2 and 3:2, respectively (Table 10). The majority were Chinese 73.7% (14/19) (Table 10).

Common ALL (C-ALL)

These leukaemic cells expressed high percentages of Ia, B4, Calla and sometimes B1 but were low for the surface membrane Ig (SmIg) marker. Since expression for cytoplasmic immunoglobulin was not done, separation into the third category, pre-B ALL, was not possible. While the majority of cases had high positivity for Ia and B4, occasionally low percentages were detected in samples that were otherwise high for the Calla marker. For example, C-71 had only 17% positivity for Ia, 27% for B4 and 55% for Calla. These results were confirmed two months later when the blood sample was again sent for IP after the patient failed to respond to cytotoxic treatment. The second readings showed a similar 13% for Ia, 23% for B4 and 60% for Calla. Thus, low positivity for the markers was not a result of bad preparation. T2 expression was seen to be low for all the cases. Only two cases (C-28 and C-58) coexpressed a myeloid marker (Table 11).

Cytochemistry tests done were on peroxidase and PAS. Peroxidase was negative for all cases except one which was positive on only a few cells. PAS was negative in only 28.4% (21/74, results of three others were unknown.) cases. (Table

Table 11: Surface marker expression, haematological diagnosis and biological features of the C-ALL subgroup.

#Pt	Sex	Age	Ethn	Sp.	Blast (%)	TWBC (cell/ul)	Pathological Report: Cytochemistry Tests Perox	PAS	Diagnosis	Immunophenotyping: Surface Marker Expression (%) Ia	B4	Calla	B1	SmIg	T2	My7	My9	Others
NC				PB	0	5,000-8,000			normal control	34	14	-	15	28	11-68	10	14	
C-1	F	2	C	BM	>90	2,700	-	coarse gran.	L1	67	58	47	30	29	-	-	-	-
C-2	F	2	C	PB	10	13,000	-	block	L1	63	60	63	55	17	4	3	-	-
C-3	F	3	C	BM	80	9,700	-	block	L2	60	73	76	-	-	-	-	-	-
C-4	F	3	M	BM	>90	10,600	-	-	L2(Rlps)	74	56	15	ND	10	-	-	-	-
C-5	F	3	M	BM	>90	27,900	-	block	L1	78	70	78	ND	-	6	-	-	-
C-6	F	3	C	BM	>90	14,100	-	-	NHL	48	92	82	99	8	-	-	-	-
C-7	F	3	C	BM	>90	7,000	-	fine+coarse	L3**	74	59	85	11	34	-	-	-	B2-
C-8	F	3	C	BM	>90	16,100	-	fine+coarse	L1,20%L2	90	81	80	8	ND	-	1	-	-
C-9	F	3	C	BM	90	5,500	-	coarse+block	NHL**	55	56	73	9	ND	28	-	-	-
C-10	F	3	C	PB	14	7,500	-	-	L2	80	83	77	ND	ND	10	-	-	-
C-11	F	3	M	BM	90	1,300	-	coarse+block	L1,20%L2**	10	42	59	ND	ND	-	ND	ND	T11-
C-12	F	4	M	BM	>90	9,500	-	coarse+block	L1	92	95	82	ND	ND	3	-	ND	-
C-13	F	4	C	PB	76	66,300	-	fine gran.	L1,20%L2	92	89	74	ND	7	-	ND	ND	-
C-14	F	4	M	BM	55	8,000	-	granular	L1(Rlps)	85	49	73	ND	36	-	3	1	-
C-15	F	4	M	BM	few	3,900	-	coarse gran.	ALL	53	41	61	15	ND	35	ND	ND	-
C-16	F	5	I	BM	>90	124,800	-	block	L1	93	90	87	ND	ND	-	-	-	-
C-17	F	5	C	PB	23	8,000	-	block	L2	57	57	39	21	ND	-	-	-	-
C-18	F	5	M	BM	>90	9,800	-	fine gran.	L1,20%L2**	70	89	80	ND	2	-	-	-	-
C-19	F	5	M	BM	>80	6,300	-	-	NHL**	72	78	85	16	-	11	-	ND	-
C-20	F	6	M	BM	90	15,400	-	gran.+block	L1	33	45	93	ND	-	4	-	-	-
C-21	F	6	C	PB	3	3,700	-	gran.+block	L1	55	53	39	ND	28	20	ND	3	T11=44
C-22	F	6	C	PB	15	4,000	-	ND	L1	54	63	63	39	21	14	ND	-	-
C-23	F	7	M	PB	29	24,300	-	block	ALL(Rlps)	89	81	90	84	-	4	2	4	-
C-24	F	9	C	BM	38		-	-	NHL	76	77	72	19	ND	-	ND	-	-
C-25	F	9	C	BM	80	206,100	-	coarse+block	ALL(Rlps)	92	88	53	ND	5	5	ND	ND	-
C-26	F	10	C	BM	80	3,000	-	coarse+block	ALL(Rlps)	65	71	72	5	-	13	10	2	-
C-27	F	11	M	BM	>80	2,600	-	fine gran.	L1	37	78	47	3	10	-	-	-	-
C-28	F	13	C	BM	90	3,200	-	-	Hybrid	91	88	84	33	4	-	41	-	-
C-29	F	13	C	PB	87	42,600	-	-	NHL	71	75	78	3	5	10	-	-	-
C-30	F	14	C	BM	>90	9,700	-	+	NHL**	-	91	88	30	-	-	5	3	-
C-31	F	16	I	BM	90	4,000	-	coarse+block	L1	82	97	92	9	ND	-	-	-	-
C-32	F	20	C	BM	90	50,700	-	block	L2	81	86	94	ND	3	3	-	-	-
C-33	F	21	M	BM	80	268,500	-	-	L2**	84	94	50	ND	ND	-	ND	ND	-
C-34	F	23	M	PB	35	81,700	-	coarse gran.	ALL(Rlps)*	92	76	79	16	2	-	3	1	-
C-35	F	24	M	BM	>90	41,400	-	-	L1**	88	98	91	ND	ND	-	-	-	-
C-36	F	25	M	PB	82	41,000	-	gran.+block	L2(Rlps)	-	42	15	ND	9	-	-	-	-
C-37	F	30	C	PB	13	12,000	-	fine gran.	ALL	76	74	58	64	3	-	5	-	-
C-38	F	40	C	PB	89	125,500	-	-	L2**	45	95	85	ND	ND	-	-	-	-

C-39	F	43	C	BM	80	2,200	-	-	L1**	93	88	88	72	17	-	-	-	-
C-40	F	44	C	BM	90	1,500	-	coarse gran.	L2	83	86	65	5	1	-	-	-	-
C-41	F	51	C	PB	81	49,300	-	coarse gran.	ALL	14	85	84	3	ND	-	-	-	-
C-42	F	69	C	PB	50	11,700	-	coarse gran.	ALL(Rlps)	94	85	80	2	20	-	1	-	-
C-43	M	9/12	C	PB	81	120,900	-	granular	L1	85	87	85	42	ND	4	ND	ND	-
C-44	M	1	M	BM	0	7,300	ND	ND	Lymphocyto	40	33	35	ND	ND	11	5	7	-
C-45	M	2	C	BM	90	37,200	-	coarse gran.	L3**	35	91	93	41	8	3	-	-	-
C-46	M	2	M	PB	78	42,200	-	-	L2	52	45	54	22	-	2	-	-	-
C-47	M	3	C	BM	)90	360,500	-	block	L1	93	86	80	70	ND	-	13	-	-
C-48	M	3	C	BM	)90	7,500	-	block	L1/L3	75	84	65	20	20	9	ND	ND	-
C-49	M	3	I	BM	)90	36,700	-	block	L1	84	76	69	ND	ND	3	ND	ND	-
C-50	M	3	C	PB	41	21,700	-	granular	L1	63	61	51	ND	15	-	-	-	-
C-51	M	3	C	BM	90	172,200	-	block	L1	95	94	95	ND	35	-	-	-	-
C-52	M	3	C	PB	78	96,500	-	-	L2**	82	92	90	ND	ND	-	-	-	-
C-53	M	3	C	PB	77	32,500	-	fine+block	L1,20%L2	52	20	52	ND	ND	14	-	-	-
C-54	M	4	I	BM	90	8,700	-	coarse+block	NHL**	87	94	72	ND	ND	2	ND	-	-
C-55	M	4	C	BM	90	7,200	-	block	L2**	94	93	97	1	-	-	ND	ND	-
C-56	M	6	C	PB	^0	2,100	-	coarse+block	L1,20%L2**	62	30	83	-	-	-	-	-	-
C-57	M	6	I	PB	NF	NF	-	coarse gran.	L2	63	68	58	17	7	17	ND	-	-
C-58	M	6	C	BM	)90	32,900	-	coarse+block	L2	47	96	83	38	ND	8	6	92	-
C-59	M	7	M	PB	67	16,900	-	block	L1,20%L2	84	85	84	ND	-	5	-	-	-
C-60	M	8	C	PB	59	9,300	-	-	L2	48	61	36	25	26	23	2	-	-
C-61	M	8	M	BM	90	188,500	-	coarse+block	L1	48	86	84	26	13	-	-	ND	-
C-62	M	8	M	PB	35	15,600	-	-	ALL	58	61	61	37	2	-	-	-	-
C-63	M	8	M	PB	6	4,600	-	-	CML b.c.*	53	61	51	ND	ND	25	10	5	-
C-64	M	8	M	PB	63	13,100	-	-	ALL(Rlps)	62	70	55	35	10	23	ND	ND	-
C-65	M	8	C	BM	80	10,000	-	gran.+block	ALL(Rlps)	84	78	84	3	ND	4	2	-	-
C-66	M	9	C	BM	)80	5,000	-	coarse+fine	L2(Rlps)	84	67	74	10	ND	-	ND	ND	-
C-67	M	9	C	BM	59	1,900	-	-	ALL**	68	71	75	ND	-	-	-	ND	-
C-68	M	10	C	BM	95	244,300	-	-	L2	77	92	92	ND	ND	-	ND	-	-
C-69	M	13	C	PB	0	2,000	-	coarse+block	L2**	72	52	62	ND	ND	-	-	-	-
C-70	M	14	C	PB	84	11,400	-	block	L1(Rlps)	75	89	87	88	-	7	-	-	-
C-71	M	15	C	PB	16	9,200	-	-	L2	17	27	55	ND	ND	10	1	-	-
C-72	M	15	M	BM	)90	5,500	-	granular	L2	28	14	95	ND	ND	1	ND	ND	-
C-73	M	20	C	PB	65	45,400	few+	fine gran.	L1**	82	82	81	ND	ND	6	-	-	-
C-74	M	26	I	PB	0	3,600	-	coarse+block	L2	70	64	-18	ND	ND	-	-	-	-
C-75	M	34	C	BM	80	4,100	-	-	L2	40	41	50	ND	ND	ND	ND	ND	-
C-76*	M	41	I	PB	50	6,800	NF	NF	?	74	49	12	12	36	15	7	ND	T6=18
C-77	M	54	C	PB	74	31,700	-	fine gran.	ALL	80	86	84	ND	3	-	3	-	-

^ Bone marrow however showed 90% blast NC - Normal Control

* Cases not admitted into UHKL

11). The M:F ratio of Calla+ patients was almost equal except in the infant group (in which both cases were males) and the adult group which had 1.8 times more females than males. Children made up the largest group with 64.9% (50/77) of patients. Chinese predominated forming 61% (47/77) of patients. They were more or less evenly distributed among the different age groups and between sexes (Table 11).

T-Cell

T lineage cells were positive for CD7 (T2), CD2 (T11) or CD7 and CD2 but negative for the non-T cell markers. In addition, six other T cell markers were included for determining the stages of T cell maturity. They were CD1 (T6), CD5 (T1), CD3 (T3), CD4 (T4), CD8 (T8) and TCR alpha-beta (WT31). The normal percentages for these markers in the peripheral blood were included in Table 12a.

Table 12a lists the T cell cases according to categories defining stages of maturation. CD5 is expressed weakly early in T cell maturation (Fowlkes and Pardoll, 1989) which may be the reason for the low positivity on samples with an early phenotype. The condition of the cells may also have been altered by pretreatments for example during cryopreservation. Occasionally, the low density expression of this antigen was detected (T-8, 13).

Before assigning a sample to a category, it is important to account for the presence of normal circulating T cells which may mask the actual phenotype of the leukaemic cell. Examples were T-11 and 15. While the percentage of blast cells of T-11 was not found, T-15 had 43%. Both samples had a significant number (12-25%) of cells positive for CD5, CD3, CD4 and CD8. CD3 and strong expression of CD5 showed these cells to be mature cells. However, these mature cells did not represent the whole population of T cells as they formed only a portion of T cells detected. T-11 had 12% of CD3, 17% of WT31 but 31% of CD7. T-15 had 25% of CD3 but 66% of CD2. The higher expression of only CD7 and CD2 showed both cell samples belonged to the second category.

The first category (I) was positive for only CD7 of which there were 10 samples, 29.4% (10/34, three samples, T-35, 36 and 37 could not be categorized because of insufficient results.). The second category (II) consisted of cells positive for both CD7 and CD2. Only six cases (17.6%) were observed. From double negative cells, a subgroup breaks off to become DN TCR gamma-delta cells. Only one case (T-17) was seen to have this phenotype (III). Although no monoclonal antibody against this T cell receptor was used, the high positivity (80%) for CD3 and the low level of WT31 implied that these mature cells expressed the other heterodimer. T-21 and 22 were differentiated from cells of category II by the expression of CD1 and were thus placed

Table 12a: Surface marker expression and biological features of the T-cell subgroup.

#Pt	Sex	Age	Eth Grp	Sp.	Blast (%)	TWBC (cell/ul)	Immunophenotyping: Surface Marker Expression (%) Ia	B4	Calla	T2	T11	T6	T1	T3	T4	T8	TCR	My7	My9	T-cell Category
NC				PB	0	5,000 -8,000	34	14	-	11-68	70	-	64	60	40	27	77	10	14	Normal
T-1	F	2	C	BM	95	352,000	-	-	-	49wk	-	-	-	-	-	-	-	-	-	I
T-2*	M	34	M	PB	87	68,200	-	-	-	92	-	-	-	-	-	-	-	-	-	I
T-3	M	38	M	PB	46	210,600	34	-	-	33wk	4	-	7	2	-	-	-	16	5	I
T-4	M	46	C	PB	92	65,300	40	1	-	84	5	-	5	5	2	3	ND	2	5	I
T-5	M	68	C	PB	88	325,600	43	1	-	92	5	-	-	5	1	-	4	-	-	I
T-6	F	27	C	PB	46	9,700	-	12	ND	80	5	-	8	2	4	4	ND	-	3	I
T-7	F	39	C	PB	33	87,000	-	-	-	91	6	-	7	3	-	2	2	-	-	I
T-8	M	17	M	PB	54	29,300	-	-	-	91	7wk	-	91wk	-	-	-	-	-	-	I
T-9	M	33	O	PB	41	85,700	42	2	7	72	10	4	8	4	2	5	6	12	9	I
T-10	M	72	C	PB	88	79,600	-	20	2	92	10	-	2	3	-	5	2	-	-	I
T-11*	M	2	C	PB	NF	NF	27	8	-	31wk	28wk	4	21	12	24	16	15	ND	ND	II
T-12	M	23	I	PB	35	108,300	69	14	-	71	42wk	4	15	9	4	6	ND	1	23	II
T-12	M	24	M	BM	80	10,100	64	4	-	79	21	-	73wk	-	1	5	7	4	-	II
T-14	M	38	C	PB	88	13,100	80	-	-	62	61	-	-	9	3	4	6	1	-	II
T-15	M	4	M	PB	43	14,000	36	17	8	28wk	66	-	22	25	21	24	ND	3	-	II
T-16	M	14	I	BM	85	118,000	30	5	-	54	58	1	1	7	11	12	-	ND	ND	II
T-17	M	19	I	BM	71	107,700	11	-	-	84	21wk	-	51wk	80	2	2	1	ND	ND	III
T-18	M	13	C	PB	68	29,900	2	-	-	9wk	55	3	-	1	39	31	-	-	-	IV
T-19	M	4	M	BM	86	373,200	3	1	ND	94	10wk	-	5	2	25	15	2	ND	ND	IV
T-20	M	4	M	PB	97	76,420	-	7	-	74	90	-	37	5	26	36	1	ND	ND	IV
T-21	F	7	O	PB	93	325,800	2	-	-	88	79	36	35wk	-	-	-	-	ND	-	V
T-22	M	16	M	PB	93	198,900	-	-	-	96	78	15	3	3	53	2	1	-	-	V
T-23	M	15	M	PB	79	233,000	-	-	-	70	88	9	25wk	2	2	86	-	ND	ND	V
T-24	M	14	I	PB	67	54,500	7	2	15	28wk	78	34	30	19	12	21	ND	11	11	VI
T-25	M	18	I	BM	90	60,600	-	-	-	40wk	90	76	-	7	56	84	4	-	-	VI
T-26	M	18	M	PB	79	99,100	-	1	ND	89	88	26	46	21	43	11	9	1	-	VI
T-27	M	25	M	BM	>80	24,200	1	1	-	74	21wk	16	60	3	60	28	4	ND	ND	VI
T-28	M	16	C	PB	NF	84,800	5	-	-	80	94	20	12	7	85	86	3	-	-	VI
T-29	M	4	C	BM	85	614,000	3	3	15	84	96	60	73	24	86	89	18	3	3	VI
T-30	M	32	I	PB	17	22,400	2	5	-	86	96	24	66	28	94	95	23	5	-	VI
T-31	M	32	C	BM	>90	22,300	-	-	-	91	95	25	85	22	84	73	94	-	-	VI
T-32	M	2	M	BM	19	30,300	-	6	-	50	61	-	35	58	30	46	41	-	2	VI
T-33	F	3	C	PB	54	29,300	27	-	-	16	55	-	13	40	86	1	11wk	-	-	VII
T-34	M	61	C	PB	33	47,500	40	-	-	21	42	13	26	49	47	8	42	36	31	VII
T-35	F	7	C	BM	40	4,900	-	-	ND	80	91	ND	ND	ND	ND	ND	ND	-	-	
T-36	M	8	M	BM	>90	294,300	2	2	-	77	33	ND	ND	ND	ND	ND	ND	-	-	
T-37*	M	15	C	PB	NF	4,200	38	-	-	81	33	ND	ND	ND	ND	ND	ND	2	-	

wk - weak NC - Normal Control TWBC - Total White Blood Cell Count

Table 12b: Haematological diagnosis of the T cell subgroup.

#Pt	Sex	Age	Eth Grp	Sp.	Blast (%)	TWBC (cells/ul)	Pathological Report: Cytochemistry Tests Perox.	PAS	APhos	Diagnosis
NC				PB	0	5,000-8,000				normal control
T-1	F	2	C	BM	95	352,000	-	fine+coarse	ND	L2**
T-2*	M	34	M	PB	87	68,200	-	+	ND	ALL
T-3	M	38	M	PB	46	210,600	few	-	ND	AML
T-4	M	46	C	PB	92	65,300	-	-	polar	NHL
T-5	M	68	C	PB	88	325,600	NF	NF	NF	?
T-6	F	27	C	PB	46	9,700	-	-	polar	L1(T)
T-7	F	39	C	PB	33	87,800	-	fine gran.	ND	L2**
T-8	M	17	M	PB	54	29,300	-	coarse	ND	L1
T-9	M	33	O	PB	41	85,700	60%	granular	ND	M4(? + lymphoma)
T-10	M	72	C	PB	88	79,600	-	block	NF	L1
T-11*	M	2	C	PB	NF	NF	NF	NF	NF	T
T-12	M	23	I	PB	35	108,300	NF	NF	NF	NHL
T-13	M	24	M	BM	80	10,100	-	fine	ND	NHL
T-14	M	38	C	PB	88	13,100	few	-	+	M4
T-15	M	4	M	PB	43	14,000	-	block	ND	L1
T-16	M	14	I	BM	85	118,000	-	coarse gran. + block	ND	L2
T-17	M	19	I	BM	71	107,700	-	granular	ND	NHL**
T-18	M	13	C	PB	68	29,900	-	+	ND	NHL
T-19	M	4	M	BM	86	373,200	-	granular	+	L2(Rlps)**
T-20	M	4	M	PB	97	76,420	-	-	polar	L1
T-21	F	7	O	PB	93	325,800	-	-	polar	T-ALL
T-22	M	16	M	PB	93	198,900	-	-	ND	L2
T-23	M	15	M	PB	79	233,000	-	-	+	T-ALL**
T-24	M	14	I	PB	67	54,500	-	-	+	ALL
T-45	M	18	I	BM	90	60,600	-	-	+	L2**
T-26	M	18	M	PB	79	99,100	-	coarse gran.	ND	NHL
T-27	M	25	M	BM	>80	24,200	-	gran.+block	polar	ALL(Rlps)
T-28	M	16	C	PB	NF	84,800	NF	NF	NF	?
T-29	M	4	C	BM	85	614,000	-	-	polar	T-ALL
T-30	M	32	I	PB	17	22,400	-	-	+	ALL
T-31	M	32	C	BM	>90	22,300	-	coarse gran.	ND	NHL
T-32	M	2	M	BM	19	30,300	-	coarse gran. + block	ND	L1**
T-33	F	3	C	PB	54	29,300	NF	NF	NF	NHL
T-34	M	61	C	PB	33	47,500	— dry tap —			NHL
T-35	F	7	C	BM	40	4,900	-	-	ND	L2
T-36	M	8	M	BM	>90	294,300	-	-	ND	L1
T-37*	M	15	C	PB	NF	4,200	NF	NF	NF	T-ALL

wk - weak NC - Normal Control

into category V. Preceding this was category IV, positive for only CD7 and CD2 and expressing relatively high levels of CD4 and CD8. Three samples (T-18, 19 and 20) had this phenotype. The lack of CD1 and also CD3 and WT31 suggested these three samples did not belong to the usual DP cells (category VI). The precursors to TCR alpha-beta cells develop from DN cells which first become immature SP (CD8/CD4) thymocytes classified here as subgroup V. Cells at this stage are still negative for CD3 and WT31. T-22 and 23 were of this category. DP cells expressed CD1, CD4 and CD8. At late stage they also expressed CD3 and WT31. Twenty four percent of cases belonged to this subgroup. Mature SP cells were categorized into group VII. Two samples, both CD4+, were collected.

None of the cells here were significantly positive for the Calla antigen. Ia was positive in 14 cases (41.2%) examined mostly in categories I and II. All had positive blasts above 10%. About 10% of T cell leukaemias were reported to express both these antigens (Foon and Todd, 1986).

Bone marrow report included tests on peroxidase, PAS and occasionally acid phosphatase (Table 12b). Three cases were positive for peroxidase, 60% in one (T-9) and a few positive blasts in the other two (T-3 and 14). All three were diagnosed as AML. IP, however, categorized them to be immature T cells. Myeloid surfacemarkers were low on all three.
PAS tested negative for 14 out of 31 cases. The other 17

cases showed a combination of fine and coarse granular to block positivity. Acid phosphatase was done on only 12 patients. All were positive.

The majority of T-ALL patients (57%, 21/37) were above 15 years of age, with a M:F ratio of about 10:1. All five adolescent patients were males. Patients aged ten and below had a more even M:F ratio which was, 7:4. None of the patients were infants (<1.5 years) (Figure 6). Chinese predominated with 14 patients (41.2%), Malays 12 (35.3%), Indians six (17.6%) while Others formed only two or 6.9% of T-ALL patients admitted in UHKL.

B-ALL

The B-ALL subgroup consisted of rare forms of B-associated malignancies (B4+, Calla-, SmIg+) including B-ALL of L3 morphology. Ten cases were phenotyped (Table 13). All showed high positivity for SmIg and low positivity for Calla except for T-4 who had an almost equal percentage of positive cells for both Calla and SmIg. However, since B1 was already high, it appeared the transition from Calla+ to SmIg+ cells was already occurring. Also, the percentage of SmIg was higher, thus the more mature stage was presenting.

Cytochemistry tests carried out on samples were negative for peroxidase. PAS was either granular positive or totally

Figure 6: Age, ethnic and sex distributions of T-ALL samples

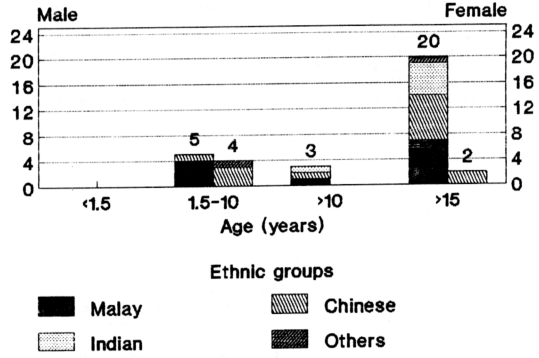


Table 13: Surface marker expression, haematological diagnosis and biological features of the B-ALL subgroup.

								Pathological Report				Immunophenotyping								
								Cytochemistry Tests				Surface Marker Expression (%)								
#Pt	Sex	Age	Ethn	Sp.	Blast (%)	TWBC (cells/ul)	Perox	PAS	APhos	Diagnosis	Ia	B4	Calla	B1	SmIg	T2	My7	My9	Others
NC				PB	0	5,000-8,000				normal control	34	14	-	15	28	11 -68	10	14	
B-1	F	3/12	M	PB	86	440,000	-	-	ND	L3	59	63	-	33	40	14	7	5	-
B-2	F	1	M	PB	13	15,200	-	granular	ND	L1,20%L2**	-	63	16	58	70	8	18	13	-
B-3	F	2	M	PB	79	5,400	-	coarse gran.	ND	L1	40	51	1	36	30	8	-	-	-
B-4	M	4	C	PB	47	9,200	-	coarse gran.	ND	L1	41	53	13	54	20	37	-	-	-
B-5	M	7	I	BM	90	20,000	-	-	ND	L3	39	64	3	66	51	10	-	-	-
B-6	F	9	C	BM	)90	26,200	-	granular	ND	L3**	89	82	-	75	83	-	-	-	B2-
B-7	F	46	C	PB	79	256,500	-	-	-	NHL	92	64	-	ND	81	-	ND	ND	-
B-8	M	52	C	BM	)20	24,700	-	-	ND	NHL**	92	95	-	87	90	-	5	2	B2=9
B-9	M	60	C	NF	NF	NF	NF	NF	NF	?	73	60	-	ND	94	-	ND	-	-
B-10*	F	70	I	PB	NF	NF	NF	NF	NF	?	70	-	-	91	80	-	-	-	-

Table 14: Surface marker expression, haematological diagnosis and biological features of the CLL subgroup.

							Pathological Report				Immunophenotyping								
							Cytochemistry Tests				Surface Marker Expression (%)								
#Pt	Sex	Age	Ethn	Sp.	Blast (%)	TWBC (cells/ul)	Perox	PAS	APhos	Diagnosis	Ia	B4	Calla	B1	SmIg	T2	My7	My9	Others
CL-1	F	A	C	BM	NF	31,800	NF	NF	NF	?	96	95	-	ND	91	-	5	-	B2-
CL-2	M	58	C	PB	6	43,200	ND	ND	ND	CLL	75	60	-	30	73	11	2	4	B2-
CL-3	M	55	C	BM	60	18,200	ND	ND	ND	CLL	74	87	-	66	82	-	-	-	-

* Cases not admitted to UHKL.

** Cases where immunophenotyping results were included in bone marrow report.

NC - Normal Control (Average of 2-4 persons)

negative. FAB classification identified only three L3.

M:F ratio among B-ALL samples was 2:3. There were again more Chinese, forming 50% of patients, followed by Malays with 30% and Indians with 20%. Age distribution, however, showed that patients were either below the age of 10 (60%) or above 40 years of age (40%).

3.2.1.2 Chronic Lymphocytic Leukaemia

Only three cases of CLL were immunophenotyped. All were mature B (Calla-, SmIg+) cells. Bone marrow reports did not include cytochemistry tests. There were one female and two males. All were adults (Table 14).

3.2.1.3 Acute Myeloid Leukaemia

Forty three cases of AMLs were immunophenotyped. Additional markers included My4, Mo1, Gp1b, Gp11b/111a and Gp111a. The identification of positive cells was made difficult by the presence of endogenous peroxidase on myeloid cells. While strong positive cells were picked out easily, weak positive was a problem. Ia, My4 and Mo1 were usually strong positive, My7 and My9 markers occasionally stained cells weakly resulting in an underestimation of positive cells. FAB classification identified 5 M1, 6 M2, 2 M3, 8 M4, 6 M5, 1 M6 and 5 M7. IP studies showed high expression of HLA-DR among M1 and M2 cases (except one, M-6). Of these, 80% (8/10) expressed My7, 67% (6/9) expressed My9 while 1/7 (14%) and 1/8 cases (13%) expressed My4 and Mo1, respectively.

Table 15: Surface marker expression, haematological diagnosis and biological features of the AML subgroup.

							Pathological Report					Immunophenotyping										
							Cytochemistry Tests					Surface Marker Expression (%)										
#Pt	Sex	Age	Eth	Sp	Blast (%)	TWBC (cells/ul)	Perox.	PAS	SE	NSE	Diagnosis	Ia	B4	Calla	T2	My7	My9	My4	Mo1	Gp1b	GpIIb/ IIIa	GpIIIa
NC				PB	0	5,000-8,000					normal control	34	14	-	11-68	10	14	17	5	-	-	ND
											?	35	22	-	43	-	ND	ND	-	14	-	-
M-1	F	9/12	M	BM	NF	12,700																
M-2	F	5	C	PB	87	88,800	-	-	ND	ND	AML**	2	2	-	7	13	98	-	-	-	-	ND
M-3	F	5	C	BM	90	78,100	)50%	-	ND	+	M4	41	-	-	16	56	83	47	ND	ND	ND	ND
M-4	F	6	M	BM	)90	71,000	few	+	ND	-	M2**	94	21	ND	4	37	37	-	4	-	-	-
M-5	F	7	C	PB	)90	7,800	-	-	ND	ND	L1	2	-	-	10	-	92	-	-	-	-	-
M-6	F	12	C	BM	)90	192,600	10%	-	ND	ND	M1	7	-	-	-	88	78	ND	ND	ND	ND	ND
M-7	F	P	C	PB	49	60,600	68%	+	ND	ND	M7	33	6	-	42	2	6	-	-	15	-	ND
M-8	F	12	C	BM	90	82,000	23%	+	+	+	M4	79	-	-	-	-	-	34	21	ND	ND	ND
M-9	F	14	I	PB	68	103,200	+	-	ND	ND	M2	97	-	-	-	70	91	36	4	ND	ND	ND
M-10	F	15	C	BM	55	28,200	+	-	ND	ND	M6**	75	8	-	10	32	82	12	2	-	-	ND
M-11	F	17	I	BM	many	11,500	-	-	ND	ND	M7**	26	7	-	6	9	-	ND	ND	14	-	50
M-12	F	19	I	BM	)90	45,000	+	-	-	+	M7	94	8	-	2	45	-	-	-	-	-	ND
M-13	F	22	C	PB	16	18,300	--- dry tap ---				CML b.c.	73	11	5	14	64	6	ND	ND	ND	ND	ND
M-14	F	25	M	BM	70	4,800	+	+	+	11	M4	29	-	-	-	-	80	ND	ND	ND	ND	ND
M-15	F	35	M	PB	57	64,000	+	-	ND	-	M5	37	4	2	6	53	51	40	42	ND	ND	ND
M-16*	F	35	C	PB	80	18,000	+	ND	ND	ND	AML	29	-	ND	-	ND	67	72	ND	-	-	-
M-17	F	40	C	BM	)80	290,400	+	-	+	-	M1	90	2	-	-	45	67	-	-	-	-	-
M-18	F	41	C	PB	86	288,300	90%	-	ND	ND	M3	-	1	-	-	-	95	-	-	ND	ND	ND
M-19	F	43	C	BM	90	51,700	50%	-	ND	ND	M4	-	-	-	-	78	66	11	9	ND	ND	ND
M-20	F	46	I	PB	34	347,400	+	-	ND	ND	M2	23	ND	ND	ND	-	-	15	-	ND	ND	ND
M-21	F	50	C	PB	47	10,800	80%	-	ND	ND	M2	87	-	-	-	69	-	ND	ND	ND	ND	ND
M-22	F	59	I	PB	93	99,600	10%	-	+	-	M1	76	-	-	-	78	-	-	-	ND	ND	ND
M-23	F	62	C	PB	71	40,500	+	-	ND	ND	M1**	60	-	-	-	47	79	-	4	-	-	ND
M-24*	F	63	M	PB	95	195,000	NF	NF	NF	NF	?	-	-	-	-	27	67	2	-	ND	ND	ND
M-25	F	69	I	BM	)80	15,200	+	+	-	-	M5	18	7	-	2	33	81	ND	ND	ND	ND	ND
M-26	F	74	M	BM	)90	240,500	20%	-	ND	ND	M1	43	-	-	-	25	72	13	28	-	-	-
M-27	F		M	PB	71	107,700	+	-	ND	ND	M4	89	-	-	-	ND	51	4	ND	ND	ND	ND
M-28	M	1	C	PB	)90	426,000	-	-	-	-	M5**	9	5	-	3	62	17	14	50	ND	ND	ND
M-29	M	2	M	BM	75	20,300	-	-	-	-	M5**	33	8	-	16	4	4	12	9	19	-	ND
M-30	M	4	C	BM	70	24,700	+	-	ND	ND	M2	45	-	-	-	18	ND	ND	ND	ND	ND	ND
M-31	M	8	M	PB	62	63,800	-	-	ND	ND	M7**	37	-	-	19	-	-	ND	ND	2	-	19
M-32	M	12	M	PB	72	89,800	-	ND	ND	ND	M2	92	-	-	-	-	80	ND	-	ND	ND	ND
M-33	M	14	C	BM			+	-	ND	ND	M3	-	ND	ND	ND	7	47	-	-	ND	ND	ND
M-34	M	18	M	BM	50	6,900	30%	-	ND	ND	AML	65	10	-	7	62	20	18	18	-	-	ND

4-35	M	20	I	BM	10	37,600	few+	-	ND	ND	CML b.c.	-	-	-	-	37	ND	ND	ND	ND	ND	ND
4-36	M	20	C	PB	75	359,200	NF	NF	ND	ND	?	53	-	-	-	-	51	ND	ND	ND	ND	-
4-37	M	24	I	PB	84	169,000	-	+	+	ND	M5	19	-	-	-	8	16	58	ND	ND	ND	ND
4-38	M	27	M	PB	66	138,000	-	+	ND	ND	M7**	68	-	-	-	5	-	4	ND	ND	ND	16
4-39	M	37	C	PB	90	7,800	20%	+	ND	ND	M4	57	-	-	-	23	-	33	21	ND	ND	ND
4-40	M	43	C	PB	68	148,400	+	+	ND	ND	M4	84	-	-	4	-	70	4	ND	ND	ND	ND
4-41	M	44	C	BM	90	386,000	-	-	+	+	M5	54	1	-	3	-	18	ND	ND	ND	ND	ND
4-42	M	63	D	BM	)90	18,800	50%	+	ND	ND	M4	73	-	-	-	33	57	ND	ND	ND	ND	ND
4-43*	M		C	PB	19	16,600	NF	NF	NF	NF	?	56	7	-	-	51	34	17	5	ND	ND	ND

* Cases not admitted in UHKL.

** Cases where immunophenotyping results were included in bone marrow reports.

M3 cases were negative for HLA-DR, My7, My4 and Mo1. Nine other AML cases were also negative for HLA-DR. Of these, two (M-2 and M-5) had phenotypes similar to M3, another two (M-19 and M-24) further expressed My7 while Mo1 was expressed on M-28, and My4 on M-37. The IP results for three others were incomplete. The diagnosis for the nine HLA-DR negative cases include AML, L1, M1, M4, 2 M5, query (?) and CML blast crisis (Table 15). M4 cases consisted of mostly HLA-DR+ cells. My4 was expressed on 60% (3/5) and Mo1 on 100% (3/3) cases tested. My7 (50%) was expressed less than My9 (71%) compared to M1 and M2s. M5 cases consisted of a more heterogenous population. Three cases were HLA-DR-, two, HLA-DR+ and the sixth case was immunophenotyped to be a M7. Of the five M7 cases, only four (M-1, M-7, M-11, M-31 and M-38) expressed megakaryoblast surface markers. A sixth sample (M-29) was also found to be positive for megakaryoblastic markers but was diagnosed as M7.

Overall M:F ratio was 1:1.7. More than half were Chinese with 52.5%, 25% Malays, 20% Indians and 2.5% Others. There were 1.7 and 3 times more females among Chinese and Indians, respectively. M:F ratio among Malays was 1:1 (Table 15).

3.3 CLINICAL PRESENTATION

Before assessing the data it should be noted that a large number of patients were referral cases from general practitioners, private medical centres or other government hospitals. A patient might be referred directly without treat-

ment, test done or diagnosis or might have been given symptomatic treatment (for example, blood transfusion) or partially treated with cytotoxics. Initial presentations may be included, otherwise only current status was mentioned.

Total white blood cell count (TWBC) was divided into three groups, $>50x10^3$ cells/ul, $10x10^3$ -$50x10^3$ cells/ul and $<10x10^3$ cells/ul (Borowitz and Falletta, 1988). Null-ALL with 21 (4/19), 37 (7/19) and 42% (8/19) (Table 10) had a similar trend of increase to C-ALL cases which had 17.3 (13/75), 34.7 (26/75) and 48.0% (36/75), for the three groups respectively (Table 11). The majority, 61% (22/36), of T cell leukaemias had TWBC of $>50x10^3$ cells/ul while 31 (11/36) and 8.3% (3/36) were at the intermediate and low range (Table 12a). B-ALL cases had 25 (2/8), 50 (4/8) and 25% (2/8) of cases in the highest, intermediate and lowest groupings, respectively (Table 13). All CLL cases had between $10x10^3$ - $50x10^3$ cells/ul. The majority of AML, 57% (24/42) had high counts, while 33 (14/42) and 10% (4/42) of AML were of intermediate and low stages, respectively (Table 15).

A total of 59 cases were collected to compare clinical features: 8 null-ALLs, 9 C-ALLs, 1 B-ALL (Table 16), 28 T-ALLs (Table 17) and 13 AMLs (Table 18).

Lymphadenopathy (LN), organomegaly of the spleen (splenomegaly) and liver (hepatomegaly) were assessed by clinicians.

Table 16: Clinical features and FBC report of the Null-ALL, C-ALL and B-ALL subgroups.

No.	#Pt	Sex	Age	Ethn	TWBC (cells/ul)	Blast (%)	Hb (g/100ml)	Plt 3 (x10/ul)	LN	Spleen (cm)	Liver (cm)	M.Mass	Others
NC		M			5,000-8,000	0	12.5 -17.5		-	-	-	-	-
NC		F			5,000-8,000	0	11.5 -15.5		-	-	-	-	-
	Null-ALL												
1	N-5	M	3	C	56,200	-	3.6	50	+	1	4	-	Lumps on head
2	N-6	M	3	C	128,000	52	4.3	166	+	2	2	-	-
3	N-10	F	9	C	3,400	-	10.0	161	+	5	5	-	Easy bruising
4	N-11	M	9	M	7,300	-	4.0	30	+	1	2	-	-
5	N-14	M	14	C	96,300	96	6.5	44	+	7	5	-	Rash, gum bleeding
6	N-15	F	18	C	14,800	13	10.1	18	-	-	2	-	-
7	N-16	F	19	M	800	-	12.9	86	+	-	2	-	Neck swelling
8	N-19	M	48	C	86,000	40	4.3	70	+	-	-	-	Died.
	C-ALL												
9	C-13	F	4	C	48,000	27	7.8	ND	+	3	2	-	-
10	C-27	F	12	M	3,400	10	8.0	78	-	1	3	-	-
11	C-32	M	19	C	37,700	41	7.6	58	-	-	-	-	-
12	C-33	F	21	M	360,000	97	8.0	37	+	-	-	-	Neck swelling
13	C-40	F	42	C	1,500	-	3.1	24	+	-	-	-	Bruise on upper arm petechiae
14	C-42	F	68	C	11,700	50	11.6	147	-	-	2	-	Pinpoint spot over both legs. Died.
15	C-48	M	3	C	7,500	-	3.1	24	+	-	3	-	Easy bruising
16	C-65	M	9	C	17,000	-	5.7	130	+	-	-	-	-
17	C-75	M	34	C	4,000	-	10.6	116	-	-	-	-	Referral
	B-ALL												
18	B-8	M	52	C	24,700	37	11.3	127	-	13	3	-	-

FBC = Full blood count.
TWBC = Total white blood cell count.
Hb = haemoglobin
Plt = platelet
LN = Lymph nodes
M. Mass = Mediastinal mass.

Table 17: Clinical features and FBC report of the T-cell subgroup.

No.	Pt	Sex	Age	Ethn	TWBC (cells/ul)	Blast (%)	Hb (g/100ml)	Plt 3 (x10/ul)	LN	Spleen (cm)	Liver (cm)	M.Mass	Others
1	T-1	F	2	C	298,200	seen	7.0	66	+	7	4	-	Petechiae. Died.
2	T-3	M	38	M	96,000	4	10.1	220	+	++	-	-	-
3	T-4	M	46	C	25,000	-	11.5	130	+	-	-	-	C/o neck swelling
4	T-5	M	68	C	274,500	95	6.8	11	+	+	+	-	-
5	T-6	F	27	C	9,700	-	8.3	106	+	ND	2	-	Multiple lumps on neck
6	T-7	F	39	C	700	9	9.6	152	+	2	6	-	C/o neck swelling
7	T-8	M	17	M	34,000	64	5.7	44	+	3	2	-	Diagnosed AMoL elsewhere
8	T-9	M	33	O	8,600	-	10.3	103	+	-	2	-	C/o swelling of neck
9	T-10	M	72	C	102,200	45	6.2	18	+	2-3	-	-	Cervical swelling
10	T-12	M	23	I	100,300	23	6.8	26	+	8	4	-	-
11	T-13	M	24	M	13,300	20	13.4	34	+	-	-	-	Swelling on neck
12	T-14	M	38	C	16,500	)90	7.1	50	+	-	3	-	Swelling of neck
13	T-15	M	4	M	14,600	?	6.9	110	+	2	3	-	-
14	T-17	M	19	I	107,700	71	?	?	+	2	3	-	Bruises on leg
15	T-18	M	14	C	29,900	68	NF	NF	NF	NF	NF	+	Skin involvement
16	T-19	M	4	M	307,200	69	7.4	25	-	7	7	-	Swelling both side of neck
18	T-22	M	16	M	198,900	84	9.9	36	+	1	4	+	-
19	T-23	M	15	M	252,000	90	12.7	10	+	+	5	+	Diagnosed AMMoL elsewhere
20	T-24	M	14	I	25,700	27	12.5	110	+	2	2	-	C/o neck swelling
21	T-25	M	18	I	43,600	4	11.7	24	+	2	5	-	CNS involvement
22	T-26	M	19	M	5,800	81	7.1	35	+	2	3	-	Multiple lump over neck
17	T-27	M	25	M	24,780	41	6.8	11	-	2	4	-	Diagnosed AML elsewhere
23	T-29	M	4	C	26,900	40	8.2	9	+	8	6	-	-
24	T-31	M	32	C	22,300	?	?	?	+	?	3	+	C/o neck swelling
25	T-32	M	2	M	30,300	19	?	?	+	3	7	-	CNS involvement
26	T-34	M	61	C	47,500	21	13.4	28	+	2	3	-	Down Syndrome
27	T-35	F	7	C	45,200	?	5.2	32	+	10	8	+	Generalized skin lesion
28	T-36	M	8	M	81,700	)90	12.2	46	+	5	3	+	Petechiae over body
													Neck swelling. Petechial rash. Died.

C/o - Complained of
AMoL - Acute Monocytic Leukaemia
AMMoL - Acute Myelo-Monocytic Leukaemia

Table 18: Clinical features and FBC report of the AML subgroup.

No.	#Pt	Sex	Age	Ethn	TWBC (cells/ul)	Blast (%)	Hb (g/100ml	Plt 3 (x10/ul)	LN	Spleen (cm)	Liver (cm)	M.Mass	Others
1	M-1	F	1/12	M	7,700	1	10.1	18	-	2	2	-	M7
2	M-3	F	5	C	71,400	75	8.8	139	+	-	-	-	Neck swell due to fall
3	M-5	F	8	C	382,000	)90	9.1	39	+	-	3	-	Petechiae + bruising
4	M-6	F	11	C	162,400		5.8	92	+	2	4	-	Easy bruising
5	M-8	F	23	C	105,000		9.1	76	+	3	4	-	Gum bleeding
6	M-10	F	15	C	18,070	seen	5.4	30	-	-	-	-	Severe anaemia. Died.
7	M-17	F	40	M	209,400	89	6.6	70	-	-	-	-	-
8	M-18	F	41	C	205,000	94	9.0	40	-	-	1	-	Cerebral infiltration
9	M-19	F	43	C	82,600		6.5	36	+	-	-	-	Relapsed. Died.
10	M-23	F	62	C	31,400	88	6.8	90	-	-	-	-	-
11	M-25	F	71	I	39,800	52	9.5	71	+	4	6	-	Neck swelling. Died.
12	M-35	M	20	I	27,600	17	7.0	40	-	-	-	-	Died
13	M-38	M	27	M	138,000	66	6.3	1158	-	16	5	-	CML b.c. Died.

b.c. - blast crisis

The presence of a mediastinal mass was revealed by chest X-Ray. Lymph nodes were divided into a few areas including the cervical, axilla and inguinal. However, owing to the inconsistency of reporting, presence of lymphadenopathy was noted with a `+' symbol. Of the eight null-ALL cases, seven (87.5%) presented with lymphadenopathy, seven (87.5%) with hepatomegaly and five (62.5%) with splenomegaly. C-ALL patients had a milder condition with 5/9 patients (55.6%) with lymphadenopathy, 4/9 (44.4%) with hepatomegaly and only 2/9 (22.2%) with splenomegaly. Twenty five (92.6%) out of 27 cases of T-ALL revealed lymphadenopathy, 21/25 (84.0%) had splenomegaly and 23 out of 27 (85.2%) had hepatomegaly. Among AML cases, 6/13 (46.2%) had lymphadenopathy, 5/13 (38.5%) had splenomegaly and 7/13 (53.8%) had hepatomegaly. Mediastinal mass was not present among the null-ALL, C-ALL and AML cases. However, it was found in 6 out of 28 (21.4%) cases of T-ALL done.

Other symptoms included petechial rashes, bruises and bleeding. One persistent complaint seen was swelling or lumps on the neck caused by swollen lymph nodes. This was most evident among the T-ALL patients with 12 (42.9%) out of 28 patients presenting with this condition. Only 1/8 (12.5%) such complain was found among the null-ALL, 1/9 (11.1%) among the C-ALL and 2/13 (15.4%) among the AML subgroup. Another notable point among the T-ALL cases was that other than the three patients with bone marrow aspirations diag-

nosed as AML cases in UHKL, another three patients were also initially diagnosed as AML elsewhere.

3.4 DNA ANALYSIS

3.4.1 Extraction of the cDNAs

Recombinant plasmids carrying the cDNAs were extracted from the various E. coli clones. The plasmids were then digested with the respective restriction enzymes to release the inserts. Figure 7 shows the various plasmids before and after restriction enzyme digestions. All the inserts were of the correct sizes as given in the references.

The inserts were then purified by electro-elution on agarose gel. Figure 8 shows the purified probes.

3.4.2 Southern Blot

Human DNAs extracted from peripheral blood or bone marrow samples were digested separately with EcoRI, BamHI, and HindIII before loading onto an agarose gel for overnight electrophoresis. Figure 9a shows an agarose gel after electrophoresis and ethidium bromide staining. After further treatment, the DNA smears were transferred overnight onto a nylon membrane by using the Southern technique. Figure 9b shows the gel after transfer and restaining with ethidium bromide.

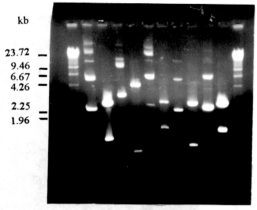


Figure 7.

Recombinant plasmid before (first lane) and after (second lane) restriction enzyme digest. Lanes 1 & 2, the alpha chain; lanes 3 & 4, beta chain; lanes 5 & 6, gamma chain; lanes 7 & 8, J delta 1; lane 9 & 10, the C delta chain. Lambda phage digested with HindIII was also included on the outermost lanes.

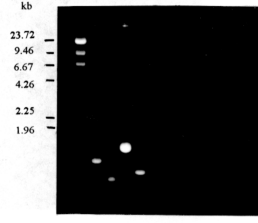


Figure 8.

Purified probes. Lane 1 - alpha chain, 1.3kb; lane 2 - beta chain, 700bp; lane 3 - gamma chain, 1.6kb; lane 4 - J delta 1, 1.0kb; and lane 5 - C delta chain, 1.5kb.

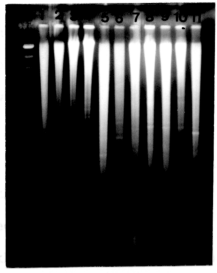


Figure 9a

Agarose gel after overnight electrophoresis of digested human DNA samples (lanes 1-7, EcoRI digest; lanes 8-11, BamHI digest) and staining with ethidium bromide.

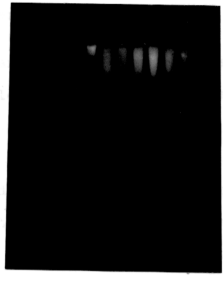


Figure 9b

The same gel after Southern transfer of the human DNA samples and staining with ethidium bromide.

3.5 GENE REARRANGEMENT ANALYSIS

3.5.1 The Human C Delta Chain

The C delta probe is a 1.5 kb cDNA fragment derived from the C delta gene which is approximately 5 kb in size (Takihara et al., 1988). EcoRI digest of the C delta gene yields three bands 3.0, 1.6 and 1.4 kb (Hara et al., 1988a; Figure 3-ai). In addition, the cDNA also contains a J segment, 3' and 5' untranslated regions (Takihara et al., 1988). The J segment runs as a 6 kb unrearranged band on EcoRI digested blots (Biondi et al., 1990). The 5' untranslated region is contained on the 6 kb fragment, while the 3' untranslated region is carried with the constant regions. The sequences encoding the J segment gene are, however, too few and the band was not always detected under high stringency washes. In cases of rearrangements to J_{delta} 1, the band carrying the J segment appears to be deleted because the 5' untranslated region is deleted in these rearrangements (Hara et al., 1988a).

EcoRI digested DNA hybridized with the probe revealed only C-delta regions, therefore no rearrangements were observed in the bands. Loss of the bands, however, is indicative of deletion of the delta locus from the chromosome. When normal peripheral blood (PB) DNA was digested with EcoRI and hybridized with the C-delta probe, the 3.0, 1.6 and 1.4 kb bands (the last two bands were observed as one) were

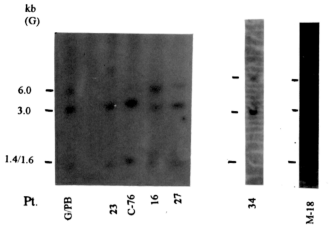
detected while the 6.0 kb band was detected only under low stringency washes (PB, Figure 10).

BamHI digestions of DNA from non-T cell lines produces two germline bands, 12 and 14 kb (Takihara et al., 1988; Figure 3-aii). D-delta3 and J-delta1 and 2 are located on the 14 kb band while the 12 kb band contains J-delta3, the constant regions and V-delta3 (Figure 3-ai,ii,iii). V-delta segments are dispersed within the locus and are interspersed between V-alpha segments (Figure 3-aiii). V-delta1 is situated a long distance 5' to the C-delta locus. V-delta2, which is closer, lies within 100 kb 5' of C-delta. V-delta3, on the other hand, lies in an inverted orientation, about 2-3 kb 3' of the C-delta segment (Figure 3-aiii). Rearrangement of V-delta3 occurs by inversion of D-delta:J-delta:C-delta leaving V-delta2 undeleted. Other unidentified rearranging regions do exist between V-delta1 and V-delta2, (Hata et al.,"1989). Casorati et al. (1989) proposed the 5'-3' order of five putative V-delta genes to be: V-delta1, V-deltax1, V-deltax2, V-delta2 and V-deltax3. Takihara et al. (1989) also identified six V-delta regions and have determined the germline bands after EcoRI, BamHI and HindIII digestions.

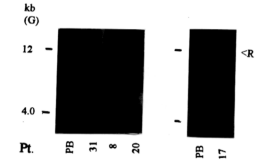
DNA analysis of PB T cells showed disappearance of the 14 kb band and diminished intensity in the 12 kb band, suggesting that the majority of these cells had undergone rearrangement

Figure 10: The human C delta-chain (cDNA)

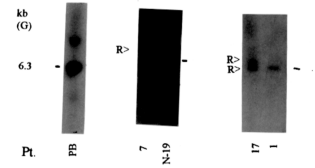
EcoRI digest



BamHI digest



HindIII digest



The large BamHI band makes it difficult to detect small changes within it. Changes in the band may result from rearrangements to J-delta3 on the 5' side or rearrangements involving V-delta3 on the 3' side of the constant regions. BamHI digested PB DNA revealed only 12 kb bands upon hybridization with the C-delta probe (Figure 10: PB). In most cases, a band of about 4 kb was also observed [PB, (G4.0); Figure 10]. This band was much reduced in intensity and probably contains the 5' or 3' untranslated region. This band was not detected on any of the leukaemic samples.

Germline DNA digested with HindIII reveals a 6.3 kb C-delta fragment corresponding to the most 3' region of C-delta (Hara et al., 1988a; Figure 3-aii). Normal peripheral blood (PB) DNA digested with HindIII revealed an intense band at approximately 6 kb (PB, Figure 10).

The C-delta probe is only able to differentiate between samples not deleted or deleted for the delta locus. Of 23 samples only 5 have deleted the delta locus. All belonged to group VI. However, T-34, a mature T cell sample was not deleted nor showed sign of rearrangement. Rearrangements observed on BamHI and HindIII fragments may be because of the presence of J or V segments. Six out of 23 DNAs digested with BamHI were rearranged. This number may be higher as it was difficult to detect small changes on the large bands (BamHI fragment). Rearranged HindIII fragments were ob-

served in 3 out of 20 samples (T-5, 7 and 17). One of these, T-17, also had a corresponding rearranged fragment on BamHI digest. All three rearrangements resulted in a 6.7 kb HindIII fragment (an additional 6.0 kb was observed in T-17). These rearrangements may or may not have involved the same V or J segments (Table 19). None of the non-T cell samples were deleted for the delta locus. Neither had they rearranged the BamHI or HindIII band. B-cell samples however tended to delete the 6.0 kb band (EcoRI digests) (Table 20).

Loss of the 6.0, 3.0 and 1.6/1.4 kb bands (EcoRI digest) in many instances did not correspond to the deletion of the whole delta locus from the chromosome, because the BamHI and HindIII fragments were still observed. It, however, corresponded to the loss of three similar bands in EcoRI digested samples hybridized with the alpha probe (discussed below).

3.5.2 The Human J Delta Probe

EcoRI digests germline DNAs to give a 6.0 kb fragment carrying the D-delta3 and J-delta1 elements (Biondi et al., 1990). Germline J-delta2 and 3 are contained on a 4.0 kb band (Hara et al., 1988a; Figure 3-aii). There is 53% similarity at the nucleotide level between J-delta1 and J-delta3 (Hata et al., 1988). EcoRI digested PB DNA hybridized to the J-delta probe did not reveal the 6.0 kb band.

Table 19: Delta, gamma, beta & alpha TCR gene rearrangement analysis of T-cells.

#Pt	C delta			J delta			Gamma		
	Eco	Bam	Hind	Eco	Bam	Hind	Eco	Bam	Hind
G(Kb)	3.0 1.6,1.4	12	6.3	6.0	14	6.0	7.2,5.0,0.5 +5.2,1.0	15,12.5 4.2,4.4,3.3	14,4.3,3.5 2.8+2.2
PB	-	G (G4.0)	G	-	G(wk)	G(wk)	-	G/C1	G/G
T-2	-	-	-	-	R10	R5	-	C2/C2	G/G
T-3	-	-	-	G	G	G	G/G	G/G	G/G
T-4	-	G	G	-	G	G	-	G/G	-
T-5	-	-	R6.7	-	-	-	-	-	-
T-7	-	G	R6.7	-	-	-	-	-	-
T-8	-	R14	G	-	-	-	G/G	-	G/G
T-9	-	G	G		R10	G/R5.0	-	G/G	G/G+#D2.2
T-10	-	G	-	-	R10/R11	-	-	C1/C2	-
T-11	-	G	G	-	G	G	G/G	C1/C1	G/G
T-12	G(G6.0)	G	-	R5.0	R10	G	G/G	C1/C1	-
T-13	-	G	-	-	-	-	-	C1/C1	-
T-14	-	-	-	G/R10	G/R10	R6.5	G/G	C1/C1	G/G
T-16	G(G6.0)	G	G	R3.0	R10	R5.0	C2/C2+#G5.2 +R2.4/R1.8	C2/C2 +R13	C2/C2+#G2.2
T-17	-	R14	R6.0/R6.7	R8.0	-	R6.5/R10	-	C1/C2	G/C2+#G2.2
T-18	-	-	-	-	D	D	G/C2	C1/C2	G/C2
T-19	-	R15	G	R3.0	-	R11	C2/C2+D5.2	C2/C2	C2/C2+D2.2
T-20	-	R14	-	-	-	-	-	-	-
T-21	-	R15	G	-	-	-	-	C2/C2	-
T-22	G(D6.0)	G	-	-	R11	-	-	C2/C2	-
T-23	-	G(wk)	G(wk)	-	-	-	-	-	-
T-24	-	G	G	R5.0	G	G	-	-	C2/C2+D2.2
T-25	D	D	D	-	-	-	C2/C2+#G5.2	C2/C2	-
T-26	-	G	G	R5.0	R20	-	C2/C2+#G5.2	C2/C2	-
T-27	G(G6.0)	R14	G	R12	R10	R8.0	C2/C2+D5.2 +R3.0	C2/C2 +R2.8	C2/C2+D2.2
T-29	D	-	D	D	-	D	-	C2/C2	-
T-30	D	D	D	-	-	D	G/C2+#G5.2	C1/C2	G/C2+#G2.2
T-31	-	D	D	-	-	-	C2/C2+#G5.2	C2/C2	-
T-32	D	D	D	-	-	-	-	-	-
T-34	G(G6.0)	G	G	G	G	G	G/G	G/G	G/G

R = rearranged G = germline D = deleted - = not done
C1 = involved C1 C2 = involved C2, deleted C1 # deleted one allele
a/b = allele 1/allele 2

Table 19: con'd

#Pt	Beta			Alpha		
	Eco	Bam	Hind	Eco	Bam	Hind
G(Kb)	11.0 4.0	24	7.5,6.0 3.3		Text	
PB	C2/C2	G	G	R1	G	-
T-2	-	-	G	R1	G	G
T-3	R8/C2	-	G/C2	R1	-	-
T-4	-	G	-	R2	G	G
T-5	-	-	-	R@	R	-
T-7	-	-	-	R3	-	-
T-8		-	-	R2	-	-
T-9	-	G	G	-	G	G
T-10	-	R15	-	R1	G	-
T-11	R2.5/C2	G	G/C2	G	-	G
T-12	-	G	-	G	G	G
T-13	-	-	-	R1	-	-
T-14	R8/R8	-	G	G	G	G
T-16	G	G	-	-	-	G
T-17	-	-	-	-	-	-
T-18	-	-	G	G	G	G
T-19	-	-	C2/C2	-	-	-
T-20	-	-	-	R1	G	-
T-21	-	-	-	-	-	-
T-22	-	R)24	-	-	-	-
T-23	-	-	-	-	-	-
T-24	-	G	-	R1	G	G+R5.8
T-25	C2/C2	-	-	-	-	-
T-26	R11/C2	-	-	R1	G	-
T-27	C2/C2	R14/15	C2/C2	-	R	R(D3.0)
T-29	-	R10	-	-	-	-
T-30	C2/C2	-	C2/C2	-	-	-
T-31	-	-	-	R3	-	-
T-32	-	-	-	R2	-	-
T-34	G	G	G	G	G	G

@ deleted 6.0, 3.0 and 1.6 kb

Table 20: Delta, gamma, beta and alpha gene rearrangement analysis of non-T cells.

No.	#Pt	C delta			J delta 1			Gamma			Beta			Alpha		
		Eco	Bam	Hind	Eco	Bam	Hind	Eco	Bam	Hind	Eco	Bam	Hind	Eco	Bam	Hind
	AML															
1	M-17	G(G6.0)	-	-	G	G	G	G/G	G/G	G/G	-	G	G	-	-	G
2	M-18	G(G6.0)	G	G	G	G	G	G/G R2.3	G/G	G/G	G	G	G	G	G	G
3	M-23	G(G6.0)	-	-	R12	R20	G	-	G/G	G/G	-	-	G	-	-	G
4	M-43	-	G	G	-	G	G	-	G/G	G/G	-	-	-	-	G	G
	C-ALL															
5	C-42	G(D6.0)	G	G	R5.0	-	-	-	-	-	-	-	-	-	-	-
6	C-32	-	-	-	-	D	D	-	C2/C2	C2/C2+D2.2 +R4.2	-	R	C2	G	-	-
7	C-45	-	-	-	-	-	G	-	-	G/G	-	-	G	-	-	-
8	C-76	G(D6.0)	G	G	-	-	D	-	C1/C1	G/G+D2.2	-	G	G	-	-	G
	Null-ALL															
9	N-19	-	G	G	-	-	-	-	-	-	-	-	-	R1	-	-
	B-ALL															
10	B-6	G(D6.0)	-	G	-	-	-	-	-	-	-	-	-	-	-	-

R = rearranged G = germline D = deleted # = deleted one allele - = not done

C1 = involved C1

least to J-delta1. Two T-cell and two AML samples maintained the 6.0 kb band (Table 20) while no 4.0 kb band was detected on any samples.

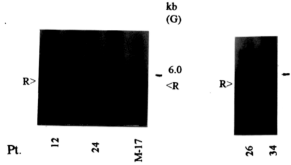
Germline DNA digested with BamHI and probed with both J-del ta1 and 2 shows a 14 kb band (Tkachuk et al., 1988; Figure 3-a). Biondi et al. (1990) reported an 18 kb band containing D-delta3, J-delta1 and J-delta2. Only a weak band was observed in DNA from PB (PB, Figure 11).

HindIII digested germline DNA produces a 6.0 kb fragment containing D-delta3, J-delta1 and a 3.0 kb fragment carrying J-delta2 when probed with MH6, a cDNA fragment which contains both J-delta1 and J-delta2 (Biondi et al., 1990; Figure 3-aii). Normal peripheral blood hybridized to the J-delta probe revealed a weak 6.0 kb band (PB, Figure 11)

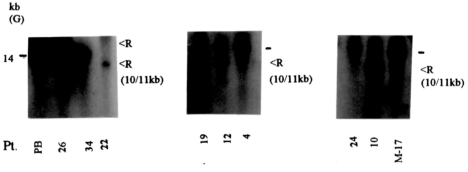
Overall results of the three restriction enzymes showed 63% of cases (12/19) had rearranged to the J-delta1 gene while three others (T-18, 29 and 30) had deleted the J-delta segment. Two of these (T-29 and 30, group VI) were also deleted for the whole delta locus (result from C-delta probe). Some early thymocytes showed no sign of rearrangement (T-3, 4 and 11), and the only other unrearranged case was T-34, a fully matured T cell (Figure 11, Table 19). The lack of rearrangement in this restriction fragment

Figure 11: The human J delta 1

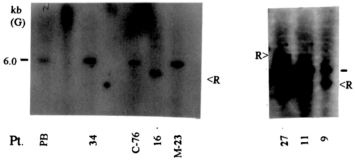
EcoRI digest



BamHI digest



HindIII digest



(among early thymocytes) does not mean that rearrangements 5' to it had not occurred (Figure 3-a) since many V and D segments are situated 5' to it. V to D rearrangements occur first in the delta gene (Fowlkes and Pardoll, 1989). Among the non T cell leukaemia cases, only one of the four AML samples was rearranged. The four C-ALL cases were either rearranged or deleted for the gene (Table 20).

Most alleles in T cell samples (9/10) had rearranged to a band of about 10 or 11 kb on BamHI digested DNAs (Table 19 and Figure 11). This 10/11 kb band was similar to the 11 and 10.5 kb bands observed by Takihara et al. (1989) on the DNA of bulk CD4-CD8- peripheral T cells similarly digested with BamHI. The sizes of rearranged restriction fragment bands on DNAs digested with the other two enzymes, appeared to be random with no similarity to the patterns reported by Griesinger et al. (1989).

3.5.3 The Human Gamma Chain

The human gamma chain cDNA contains C-gamma1, J-gammaP and V-gammaII (Yoshikai et al., 1987) or V-gamma9 (Lefranc et al., 1986a). Bone marrow DNA digested with EcoRI and probed with the human gamma chain cDNA revealed six bands. They were 7.2, 5.0, 2.5, 2.3, 2.0 and 0.6 kb (Yoshikai et al., 1987). The 7.2 and 5.0 kb bands contain the first exon of C-gamma2 and C-gamma1, respectively (Murre et al., 1985) while the second and third exons are located on the smaller fragments (Figure 3-bi). The two constant regions are very

similar to each other having 97 and 94% homology at the nucleotide and deduced amino acid levels (Yoshikai et al., 1987). Thus, the C-gamma2 fragments were also detected on the blots. V-gammaII is located on a 5.2 kb fragment (Chen et al., 1988; Figure 3-bii) overlapping the largest C-gamma1 germline fragment. As a result, an intense band was observed at about the 5 kb position (Figure 12). JP (J1.2) differs markedly from the other joining regions. It runs as a 1.0 kb band on EcoRI digested blots (Casorati et al., 1989; Figure 3-biii).

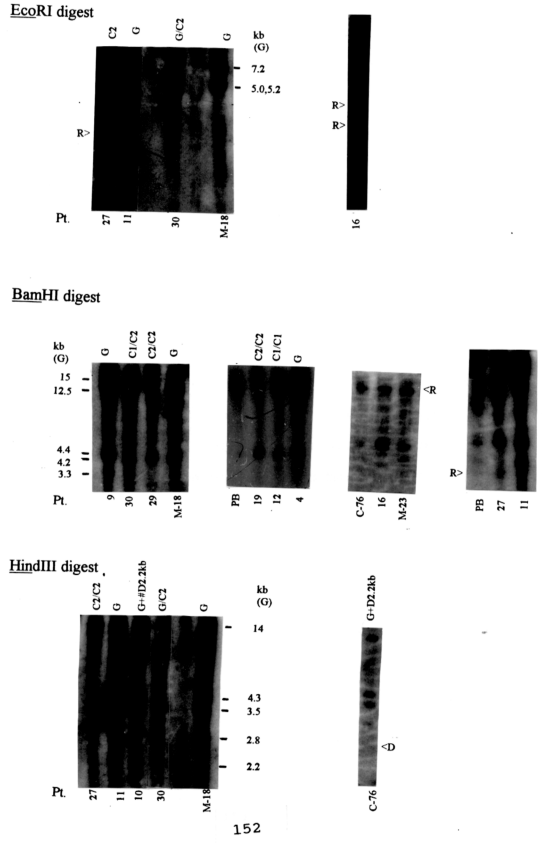
VJ recombinational events were not detected in EcoRI digests because of the presence of EcoRI sites between each of the J segments (Figure 3-biii). Rearrangement to the C-gamma2 region, however, was noted by the deletion of the 5.0 kb band (Greenberg et al., 1986). Detecting this deletions was however, not easy owing to the presence of the 5.2 kb band. The relative intensity of the 7.2 to 5.0 kb band was indicative of the number of alleles present. Two patterns of rearrangements may be detected, germline (G) when all bands were present and C2 when the 5.0 kb band was lost from the blot. Since rearrangement to C-gamma1 may be confused by the presence of the V9 fragment, C1 rearrangements were confirmed by other restriction enzyme digests. Germline, rearrangement and deletion of the V9 or JP fragment were listed as G, R and D (Table 19). No PB results were obtained from EcoRI digest. In most of the samples here, the

smaller fragments could not be detected may be because of high stringency washes and the low number of recombining sequences (Figure 12).

Bone marrow DNA digested with BamHI and probed with the human gamma chain cDNA revealed at least five germline bands (Yoshikai et al., 1987). A BamHI site is located in the middle of the first and second exons of the constant regions, and this results in the exons splitting upon BamHI digestion (Murre et al., 1985). The 5' region of the first exon of C-gamma1 is carried on a 15 kb band. The 3' end of the first exon and the 5' end of the second exon are carried on a 3.3 kb band. Similarly, the 5' end of the first exon of C-gamma2 is carried on the 12.5 kb band. This band also carries the 3' end of C-gamma1 (Davey et al., 1986; Sangster et al., 1986). Additional BamHI sites further 3' split the second and third exons of the second constant region into smaller regions including a band of about 4 kb (Sangster et al., 1986; Figure 3-bi). [Weak and intense bands existed at about 4.4 and 4.2 kb (Figure 12).] The JP gene is located on the 15 kb band. A BamHI site is located about 11 kb 5' to V-gamma9 (Lefranc et al., 1986a; Figure 3-bii) therefore the germline band probably migrates with the intense 15 and 12.5 kb bands or may be in the 4 kb band.

VJ recombinations involving C-gamma1 (C1) resulted in rearrangement of the 15 kb band leaving the rest in germline. Rearrangement to JP1 or JP resulted in JP being rearranged,

Figure 12: The human gamma-chain (peripheral blood cDNA)



whereas rearrangement to J1 resulted in JP being deleted (Figure 3-biii). Involvement of C-gamma2 (C2) resulted in deletion of the 15 and 3.3 bands and rearrangement of the 12.5 kb band. Recombination of any V-gammaI segments resulted in the deletion of V9. Recombination with V-gamma subgroups 3' to V9 left V9 in germline (Figures 3-bii and 12).

Hybridization of the gamma probe with HindIII digested normal PB DNA reveals five bands at approximately 15, 4.0, 3.5, 2.8, and 2.2 kb. The 2.8 and 3.5 kb bands contain the first exon of C-gamma1 and C-gamma2, respectively. Therefore, deletion of the 5.0 kb band in an EcoRI digested sample also result in deletion of the 2.8 kb band (Murre et al., 1985). The second and third exon of C-gamma1 may be located on another 2.8 and a 4.3 kb band (Figure 3-bi) while similar regions of C-gamma2 may possibly be on the 14 kb band. V-gamma9 runs as a 2.2 kb band (Chen et al., 1988). J-gammaP may be located on a fragment of about 1.7 kb (Figure 3-biv).

Since a HindIII site exists between the J and C regions, only rearrangements to the second region were detected (C2). Germline, rearranged and deletion of the V9 and JP fragments were listed as G, R, and D of the relevant sizes (Tables 19 and 20). C-76 lost only band 2.2. A weakening of the 4 kb band in its BamHI digested DNA suggests V9 may be located here (Table 20, Figure 12).

extent of rearrangement in the gamma gene locus. Results from other restriction enzymes, however, provided a clearer picture when necessary. Overall, rearrangements in 24 samples were observed (Table 19). Three were at germline (T-3, 4 and 34). Four cases involved C-gamma1 alone while 15 others involved C-gamma2. Of 12 alleles rearranged to C1, 10 were from groups I, II and III. Involvement of the V9 segment in gamma gene rearrangments was observed in two patients (T-16 and 27); seen as deletion of the the germline bands (on different enzyme digests) and formation of new bands. The gamma gene of T-16 appeared to have used V9 and joined it to the JP segment because a 2.4 kb rearranged band was observed under EcoRI digest similar to that reported by Leber et al. (1989). The HindIII equivalent however, was not detected. Deletion of V9 on one or both alleles was found in eight patients. Seven others maintained V9 at germline (Table 19, Figure 12).

All four AML samples maintained the gamma gene at germline. C-ALL samples showed rearrangements involving V9 in one of the samples (C-32, Table 20).

3.5.4 The Human Beta Chain

The beta cDNA used here was constructed from RNA extracted from the T-cell line Jurkat (Yoshikai et al., 1984). It contains a J and C region. There are two constant regions in the beta gene, C-beta1 and C-beta2, which are very

similar in sequence. A small cluster of joining segments is located about 5 kb upstream to each constant region. J-beta1 contains six functional J gene segments while J-beta2 contains seven functional J gene segments (Toyonaga et al., 1985; Figure 3-c). Consensus sequence between the two J regions is about 46% (Toyonaga et al., 1985). Either of the C-beta probe will hybridize with the other constant region on a blot, but each J-beta segment will only hybridize to its own band (Yoshikai et al., 1984). While the constant region fragments were identified on the blot, J fragments were not easily seen, may be because the probe contained only one J segment which was made up of 48 nucleotides.

Germline DNA cleaved with EcoRI and probed with a constant region reveals two bands, an 11 kb band that contains C-beta1:J-beta1 and a 4 kb band which contains C-beta2. Rearrangements involving C-beta1 result in the loss of the 11 kb band and the appearance of a new band. If the other allele remains in germline, then a single, but one-half dose, germline band will be retained (Cossman and Uppenkamp, 1988). A DJ-beta1 type of rearrangement will also result in rearrangement of the 11 kb band. When C-beta2 is rearranged, the C-beta1 region is lost from the genome, thus the 11 kb band is not seen on a Southern blot (Slingerland et al., 1988). Rearrangement involving the J-beta2:C-beta2 regions will not be detected in the EcoRI digest because an EcoRI recognition site is present between J-beta2 and C-beta2 (Figure 3-c). In polyclonal T cell samples their

rearranged TCR genes resulted in a diminution in dose of the 11 kb EcoRI fragment. The 4 kb band remains unaltered. However, no non-germline bands are identified. Highly polyclonal T cells such as those in normal peripheral blood or lymphoid tissues contain a wide range of restriction fragment sizes and none are detectable by Southern blot (Cossman and Uppenkamp, 1988). No PB results were obtained.

The J-beta1, C-beta1, J-beta2 and C-beta2 regions are contained on a 23 kb BamHI fragment in germline DNA (Figure 3-c). Rearrangements involving either the C-beta1 or C-beta2 regions will result in the loss of the 23 kb band and the appearance of a new band. However, owing to the large size of the fragment and the fact that DNA is separated on an agarose gel in a logarithmic manner, small changes in the size of the fragment may be missed (Slingerland et al., 1988). BamHI digested PB DNA samples maintained a weak band at germline (Table 19).

Probing of HindIII digested DNA with a constant region probe resulted in three germline bands, a 3.3 kb band that contains C-beta1, a 7.5 kb band that contains J-beta2 and the 5' end of C-beta2 (J-beta2:C-beta2) and a 6.0 kb band that contains the 3' end of C-beta2. A DJ-beta1 rearrangement will not affect the germline configuration but a DJ-beta2 type of rearrangement will result in rearrangement of the 7.5 kb band without affecting the other two bands. A V

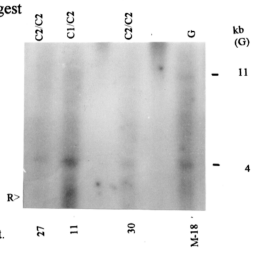
segment rearranged to the C-beta2 complex results in deletion of the fragment bearing C-beta1 and a change in size of the fragment bearing J-beta2:C-beta2 (Sangster et al., 1986 ; Figure 3-c). A complete though weak germline pattern was observed on PB samples (Table 19).

Very few complete results were obtained for this probe. There was difficulty in hybridizing the probe to the samples resulting in indistinct bands. Furthermore expected band sizes were not obtained especially on EcoRI digests. Cossman and Uppenkamp (1988) warned of a relatively resistant EcoRI restriction site just 5' of the C-beta2 region which results in an 8.5 kb fragment when not cleaved. In some individuals, this band (8.0 kb) is due to genetic polymorphism (Waldmann et al., 1985). The bands observed here were not of a similar size to those reported above and the results were thus excluded.

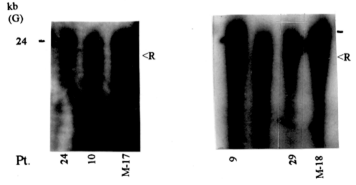
Only one sample (T-34) was in germline for the beta gene. Eleven other cases showed signs of rearrangement for the beta gene. They ranged from immature (group I) to the most mature phenotypes (Table 19). C-beta2 involvement (C2) was observed in early cells (groups I and II) but occurred on only one of two alleles (the other having rearranged to C-beta1). Mature categories (4/5 DNA samples, group IV onwards), however, rearranged C-beta2 on both alleles. The sizes of rearranged fragments appeared to be random. The status of seven others was uncertain because of incomplete

Figure 13: The human beta-chain

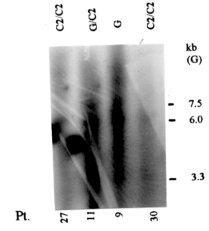
EcoRI digest



BamHI digest



HindIII digest



data since the one or two restriction digests obtained were in germline (Table 19, Figure 13).

None of the results on AML samples had any sign of rearrangement. C-ALL samples included a rearranged sample and two others with only results of germline configurations (Table 20).

3.5.5 The Human Alpha Chain, pY14

The alpha probe used here contains a V, J and C segment. The entire pY14 insert hybridizes to ~10 fragments when genomic DNA is digested with EcoRI, BamHI and HindIII. Only one or two of these bands can be assigned to the C and J sequences. The rest correspond to fragments homologous with V gene segments (Yoshikai et al., 1985). V-alpha probes from 9 different subfamilies revealed that there are at least 55 bands that can cross hybridize with these probes (Kronenberg et al., 1986). The J segment in pY14 appears to be the most 3' J segment which lies ~4 kb upstream to the C-alpha gene (Yoshikai et al., 1985; Figure 3-d).

Both the C region and J segment are located on the same EcoRI fragment and seen as a single band on EcoRI digests (Yoshikai et al., 1985). Studies using probes derived from the J regions (which extend over 50 kb) revealed 2.3 (J-alphaB), 4.7 (J-alphaC) and 8.3 kb (J-alphaD) bands on blots containing EcoRI digested DNA (Hara et al., 1987). Other

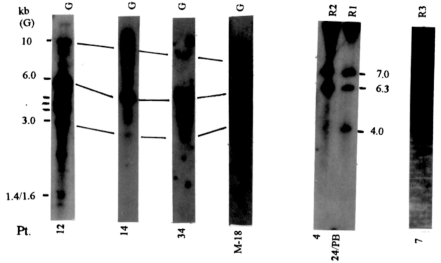
probes used include J-alphaA, J-alphaE, J-alphaF and J-alphaG (Figure 3-d). Germline samples had intense fragments at about 10, 6.0, 4.6, 4.4, 4.2 and 3.0 kb (Figure 14). Overlapping bands may also exist because some bands were more intense than others. Weak bands were observed at >24, 6.5, 8.5, 2.8 and 1.6 kb. PB samples had most of the bands deleted leaving only three rearranged bands at 7.0, 6.3 and 4.0 kb (PB, Figure 14).

The C-alpha region is excised as a 5.7 kb band on BamHI digested germline samples while the J segment is found on a 3.6 kb band (Yoshikai et al., 1985). A BamHI site is situated between the C and J regions (Figure 3-di), thus rearrangements 5' to the C region were not detected. The J alpha probe detects a 3.6 kb band, purportedly the same 3.6 kb above since the probe is obtained from a region 1 kb 5' to the constant region (Sangster et al., 1988). J-alphaE hybridizes to two bands, 6.4 and 3.2 kb, while J-alphaF and J-alphaG are contained on a 12 and 11 kb band, respectively (Hara et al., 1988b). Intense germline bands were found at a band larger than 24, 12, 10, 9.5 and 2.5 kb. In addition, weak bands at 8.5 kb, 6 to 7 bands at 6 to 7.5 kb and another band at 4.5 kb were also seen. PB samples maintained the germline configuration (Figure 14).

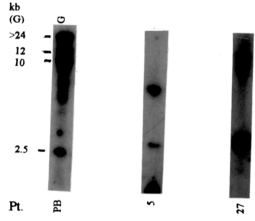
HindIII digests the C-alpha region into two bands while the J segment is contained on a single band (Yoshikai et al., 1985; Figure 3-di). Similar to BamHI, a HindIII site is

Figure 14: The human alpha-chain (pY14)

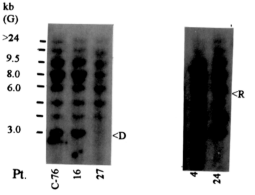
EcoRI digest



BamHI digest



HindIII digest



located between the J and C region. Therefore any rearrangements 5' to the C region were again not revealed.

Researchers found germline bands of 5.3, 1.7 and 0.7 kb with J-alphaC, 4.8 kb with J-alphaD (Hara et al., 1987) 3.4 and 4.8 kb with J-alphaE, 1.5 kb with J-alphaF and 7.6, 3.8 and 1.8 kb with J-alphaG (Hara et al., 1988b). More than 10 germline bands were observed on samples here. Strong bands appeared at >24, 15, 9.5, 8.0, 6.0, 5.0, 4.3 and 3.0 kb. PB samples also maintained these bands weakly.

Most of the rearrangements occurred on EcoRI digested samples. The type most frequently observed, designated R1, was similar to that of PB samples, that is, consisting of bands 7.0, 6.3 and 4.0 kb (Figure 14). Other patterns observed were mainly R2 (which is an R1 without the 4.0 kb band) and R3 (again similar to R1 except that it had lost the 7.0 kb band). On EcoRI germline samples, three bands, 6.0, 3.0 and 1.6 kb, appeared to correspond exactly to the three germline bands on EcoRI digested DNA hybridized with the C-delta probe. While the 6.0 kb band (containing a J segment) was weak on blots hybridized with the C-delta probe, it reacted strongly with the alpha probe. The J segment on the C-delta probe may actually carry a small portion of a J-alpha segment or cross hybridizing segments. The other two bands were weak bands on blots tested with the alpha cDNA. Only a few samples (T-11, 12, 14, 18, 34, M-18 and C-32; Tables 19 and 20) maintained the germline configuration. Of

these, T-12, 34 and M-18 were also in germline when probed with C-delta. The results for the other samples were not obtained (Tables 19 and 20).

At one stage of gene rearrangement, the 6.0, 3.0 and 1.6 kb bands could no longer be detected on the blots, even though the delta locus was not deleted from the chromosome, as evidenced by the presence of bands on other restriction enzyme digests (T-4, 5, 7, 8, 10, 13, 20, 24, 26, and N-19). At the same time, each of these samples lost the three bands on the alpha locus as shown upon hybridization with the alpha probe (Table 19). Loss of the 3.0 and 1.6 kb bands may be the result of rearrangement into smaller fragments not detectable on the blot. At least one of the V segments of the delta locus (V-delta1) is situated between V-alpha regions (Hata et al., 1989). Rearrangement of this V segment results in deletion of at least one of the V-alpha segment (Figure 3-aiii) and results in rearrangement of the alpha locus. Furthermore, V-delta1 has been identified to be one of the more frequently used V-delta segments (Casorati et al., 1989; Griesinger et al., 1989). True VJ recombination on the alpha locus results in deletion of the delta locus from the chromosome.

Of five samples deleted for the delta locus only two corresponding results were obtained with the alpha probe, that is an R3 and R2 for T-31 and T-32, respectively (Table 19).

Owing to the presence of overlapping bands, rearrangement of any single one band is impossible to detect. Only 2/15 rearrangements were observed in BamHI digest (T-5 and 27) and 2/16 in HindIII digest (T-24 and 27).

No rearrangement was observed in AML patients. Very little result was obtained on EcoRI digest. Neither was there any rearranged bands in C-ALL samples. The single null-ALL sample had a R1 rearrangement on the EcoRI blot (Table 20).